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Acute and Chronic Toxicity to the Fathead Minnow, 'Pimephales promelas', of Effluents from a Treatment Plant Receiving Dyes and Pigments Processing Wastewater

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by

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ABSTRACT

Ninety-six hour, static acute, and continuous-flow early life-stage (ELS) chronic toxicity tests, using the fathead minnow (Pimephales promelas), were conducted to evaluate the toxicity of effluents from a pilot scale activated sludge treatment system receiving dyes and pigments wastewater. In the first test with a powdered activated carbon (PAC) dosage of 1800 mg/litre of effluent, survival was the most sensitive measure of effect in the ELS test. The highest effluent concentration tested (10 percent) was lethal to the embryos and larvae. No adverse effect on survival or growth were observed at lower effluent concentrations (5.0 - 0.63 percent). The maximum acceptable toxicant (effluent) concentration (MATC) was between 10 and 5 percent. Ten 96-h median lethal concentration (LC50) values from static acute tests ranged from 12.9 to greater than 80 percent. In the second test at a PAC dosage of 1000 mg/litre of effluent, growth was the most sensitive measure of effect in the ELS test. Larval growth was significantly reduced at the highest effluent concentration tested (20 percent). The MATC was between 20 and 10 percent. Seven 96-h LC50s from static acute tests ranged from 28.3 to greater than 80 percent. The effluent from the treatment system receiving the higher PAC dosage of 1800 mg/litre was more toxic than the effluent from the treatment system receiving the lower PAC dosage of 1000 mg/litre. However, the influent to the treatment system receiving the higher level of PAC also was more acutely toxic.

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INTRODUCTION

Some Regions and States have been using toxicity test requirements or toxicity based effluent limitations in National Pollutant Discharge Elimination System (NPDES) permits. Recently the Environmental Protection Agency (EPA) has issued a policy statement that the EPA and the states may impose permit limits on effluent toxicity and may require a NPDES permittee to conduct a toxicity reduction evaluation (Federal Register on March 9, 1984, Vol. 49 (48) pp. 9017). A variety of fish toxicity tests has been developed and used to characterize the toxicity and the variability of toxicity of industrial and municipal wastewaters. The acute toxicity test is a quick and economical approach for evaluating the toxicity of complex wastewaters (1-3). Chronic toxicity tests integrate the combined effects of a complex wastewater that vary with components and concentrations and thus are effective in evaluating the long term effects of an effluent.

Recently, more rapid toxicity tests are being developed and used to estimate chronic toxicity. Birge and Black (4) conducted short term embryo larval tests of industrial and municipal effluents of which ten were final NPDES effluents discharged to receiving waters. They concluded that these tests provided a sensitive, reliable, and economical means of quantifying the chronic toxicity of complex effluents. Norberg and Mount (5) have developed and used a seven day subchronic test using newly hatched fathead minnow larvae as cost effective estimate of chronic toxicity of complex effluents.

The Industrial Environmental Research Laboratory in Cincinnati, Ohio (IERL-CI) has the responsibility for conducting inhouse and extramural research and development studies on wastewater treatment processes for a number of major industries. One of the inhouse studies was designed to evaluate the treatability of a dyes and pigments processing wastewater using an activated sludge (AS) process both with and without the addition of powdered activated carbon (PAC). The engineering design and operation of these activated sludge pilot plants are described in Shaul et al (6,7). The activated carbon and activated sludge treatment of dyes and pigments processing water are described by Shaul et al (7). The Newtown Fish Toxiciology Station (NFTS) is cooperatively involved with IERL-CI to evaluate the effectiveness of wastewater treatment processes in the removal of toxicity to aquatic life and to develop methods for evaluating technologies for reducing the toxicity of wastewaters to aquatic life.

The purpose of the present study was to investigate the acute and chronic toxicity of the effluent from an activated sludge treatment with powered activated carbon addition of a dyes and pigments processing wastewaters. Effluents from two AS/PAC pilot plants operated in a plug flow mode were evaluated. Data were collected in order to assess the effects of two PAC dosages (1800 and 1000 mg/litre of effluent) to the treatment system on the chronic toxicity of the treated wastewater. Chronic toxicity was determined on the basis of early life-stage (ELS) toxicity tests initiated with fathead minnow eggs that were less than 24-h old. The effects on embryos and survival and growth of the larvae were measured and used to estimate chronic values. Acute toxicity tests were conducted to evaluate effluent variability and to estimate acute-chronic ratios.

METHODS

Effluent and Dilution Water

The wastewater for the pilot plant study was collected approximately every two weeks from a municipal sewer downstream from the discharge of a dyes and pigments processing plant and transported to the EPA's Test and Evaluation (T&E) Facility. Further details of this wastewater and pilot plant operation are given by Shaul et al (6,7). The two effluents used in the present study are listed (7) as Segment 1, Phase 2, AS/PAC and Segment 1, Phase 3, AS/PAC, hereafter referred to as Phase 2 and Phase 3, respectively. The physical and chemical characteristics of these effluents are also given in that report. The 24-h composite samples of effluent from these two pilot treatment systems were transported daily to the NFTS for the toxicity tests. These samples were used to supply the test system within 12 hours after collection.

The dilution water was a constant quality mixture of spring water and carbon-filtered, demineralized Cincinnati tap water that was aerated in a covered, concrete reservoir and had a mean retention time of about 14 days. This dilution water had a mean alkalinity and hardness value of 179 and 207 mg/per litre (as $CaCO_3$), respectively. Dissolved oxygen ranged from 8.3 to 8.5 mg/litre and pH ranged from 8.2 to 8.4.

Chronic Exposure System

The tests were conducted with a continuous flow, mini-diluter system (8) which delivered five effluent concentrations and a control to four replicate exposure chambers. These glass chambers were 7 cm wide x 19 cm long x 9 cm high and were calibrated to contain 500 ml of test water. The flow rate to each exposure chamber was 12.5 ml per minute. Fluorescent tubes were used to provide an automatically controlled 16-h on, 8-h off photoperiod.

The mini-diluter was calibrated to give a dilution factor of 0.5, and the high test concentrations in the Phase 2 and Phase 3 tests were 10 and 20 percent, respectively. A 250-litre polyethylene tank was used to contain about 190 literes of the high test concentrations of effluent. This solution which was prepared with control dilution water was renewed daily using the preceeding 24-h composite sample. This high test concentration was delivered by a metering pump calibrated to deliver 100 ml per minute to the toxicant cell of the diluter. The pumping rate was checked daily by timing a 60 second flow into a graduate cylinder and by calculating the pumping rate from the volume used at each daily change of the high test concentration. For the Phase 2 ELS study the 60 second flow had a mean value of 99.3 ml per minute and a range of 98 to 101 ml per minute. The calculated daily rate had a mean value of 99.3 ml per minute and a range of 98.1 to 100.7 ml per minute. For the Phase 3 ELS study corresponding values were 100.3 ml per minute with a range of 100 to 101 ml per minute and a calculated daily rate of 100.2 ml per minute with a range of 99.6 to 102.5 ml per minute. The diluter operation and evaluation was similar to that given by Benoit et al (8).

Chronic Biological System

In general, the methodology of the ELS exposures was similar to that recommended by the American Society for Testing Materials (9). The tests were initiated with 12 hours old embryos from four separate spawns from the fathead minnow stock culture unit maintained at the NFTS. Individual embryos were placed in small beakers and the embryos then randomly assigned to the twenty-four test chambers. Because of a shortage of embryos only twenty were used in each cup for the first test. Twenty-five embryos per cup were used for the second test. Embryos were incubated in vertically oscillating 120 ml glass jars with 40 mesh nylon screen bottoms. On day one after beginning the exposure embryos were counted and nonviable eggs removed with the aid of a dissecting microscope. For the Phase 2 test, except for the high concentration, the embryos were thinned on day two to 15 embryos per cup with no additional thinning when the larvae were removed from the cup and placed in the exposure chambers. For the Phase 3 test upon completion of hatch, the total number of larvae in each cup was counted and 15 larvae were then randomly placed back into their respective exposure chamber. For both tests hatching was completed in five days.

Equal amounts of live San Francisco Bay brine shrimp nauplii 24 hours old were fed to excess three times daily at 4-5 hour intervals so that live brine shrimp were available to the larvae during the daylight hours from the time of the first daily feeding. Fish were not fed on the day that the test was terminated. Exposure chambers were cleaned daily with a large pipette using a safety pipet filler. The Phase 2 test was

terminated 28 days after hatch, and because effluent was not available, the Phase 3 test was terminated 21 days after hatch. After the exposure each fish was measured for length and weighed to the nearest milligram for blotted dry, wet weight.

Chronic Chemical Conditions

Routine chemical analyses of the test water were made weekly (10) with dissolved oxygen and pH measurements being made in all chambers. In the Phase 2 chronic test dissolved oxygen had a mean value of 6.7 mg per litre and ranged from 4.1 to 8.3 mg per litre. The pH ranged from 7.8 to 8.5. Alkalinity and hardness ranged from 164 to 254 mg per litre and from 190 to 298 mg per litre (as $CaCO_3$), respectively. In the Phase 3 test dissolved oxygen had a mean value of 7.0 mg per litre and ranged from 5.6 to 8.4 mg per litre. The pH ranged from 7.7 to 8.4. Alkalinity and hardness ranged from 166 to 280 per litre and from 198 to 396 mg per litre (as $CaCO_3$), respectively. The alkalinity, hardness, and pH were higher in the higher test concentrations. The test temperature for both test was $25 \pm 2^{o}C$.

Acute Toxicity

In general the acute test methodology was similar to that recommended by Peltier (3). These static acute tests were conducted at $25^{o}C$ with fathead minnows from the NFTS culture unit. These fish were from four to six weeks old and ranged from 12 to 18 mm in total length . Ten fish were used in each test concentration and control. Test volume was three liters, and the concentration interval was 50 percent. The effluent

samples and dilution water were the same as that used in the chronic exposure. The tests were initiated at three or four day intervals with ten acute tests for the Phase 2 chronic study and seven tests for the Phase 3 study.

Data Analysis

The LC50 values for the acute toxicity tests were determined by the moving average-angle method (11). Embryo survival, larval survival and the weight of the individual fish alive at the termination of the test were subjected to one-way analysis of variance (P=0.05) and, when significant, to Dunnett's one-sided comparison of concentration means to control means (12). Percentage survival data were transformed using arcsin transformations. The weights of individual fish in replicate exposure chambers were pooled and used as one group of observations.

RESULTS

Phase 2 Effluent

Survival of embryos at test day two ranged from 90 to 95 percent and was not related to effluent concentration (Table 1). However, most of the embryos in the high test concentration (10 percent) were abnormal. The number of embryos in this concentration was not thinned at test day two and there was only 14 percent survival at hatch. This low survival was significantly (P=0.05) less than all other effluent concentrations and control. All of these larvae were grossly abnormal, and all died within two days after hatching. Survival to hatch of the fish in the other effluent concentrations and control was 100 percent, and all of these larvae appeared normal. There was only a little additional mortality of larvae from hatch to the termination of the test. Survival after test day two ranged from 93 to 97 percent, and the statistical analysis (P=0.05) indicated that there were no significant differences in survival of fish in any of the four lower effluent concentrations and control.

Growth of the fish was not reduced at any effluent concentration (Table 1). Although the mean weight of the fish in the two high sublethal effluent concentrations was less than that in the two low concentrations and control, there were no significant differences (P=0.05) between effluent concentrations and control. The mean weight of the surviving fish ranged from 100 to 116 mg.

Survival of the juvenile fish in the ten acute static toxicity tests is shown in Table 2. The 96-hour LC_{50} values ranged from 12.9 to 80 percent effluent and with two exceptions the effluent concentrations gave an all-or-none response in terms of survival. Although not shown in the table, eight of the ten LC_{50} values were the same at both 24 and 96 hour exposure. Combining the survival data at each concentration from all tests, the calculated 96-h LC_{50} value is 31.2 percent. At the high lethal concentration of 10 percent in the ELS study only one fish of 100 animals was killed in the acute tests.

Phase 3 Effluent

Survival of the embryos and larvae for this ELS exposure is shown in Table 3. The embryos in this exposure were not thinned until hatching was complete. Survival of these embryos to hatching ranged from 90 to 94 percent and the statistical analysis (P=0.05) indicated that survival at hatch was not related to effluent concentration. Survival of the larvae ranged from 88 percent in the high effluent concentration to 100 percent in the low concentration. The statistical analysis indicated that there was no significant differences (P=0.05) in survival of fish in any effluent concentration and control.

Growth of the fish exposed to the high effluent concentration (20 percent) was reduced after 21 days of exposure. The mean weight of the fish exposed to this high concentration was only 39 percent of the mean weight of control fish and was significantly less (P=0.05) than that of

the control fish (Table 3). The mean weight of the fish in the other effluent concentrations was not significantly (P=0.05) different than that of control fish. The final mean weight of these fish exposed for 21 days ranged from 86 to 106 mg.

The results of the seven static acute exposures are shown in Table 4. The 96-h LC_{50} values ranged from 28.3 to 100 percent. Only five of the seven acute exposures included 100 percent effluent, and in these five concentrations 60 percent of the animals survived. All tests included 80 percent effluent and in this concentration there was 57 percent survival. Except for one concentration in one test, the effluent concentrations gave an all-or-none response in terms of survival. There was no change in LC_{50} values from 24 to 96 hours exposure in all but one test. At the test concentration of 20 percent effluent there were no adverse effects on survival in the ELS study, and there was 100 percent survival of juveniles in the acute test.

Chemical Measurements

The following chemical results were found by the University of Cincinnati following an EPA approved quality assurance/quality control plan. During the time of the Phase 2 study, ten composite samples of the effluent were measured for NH_3-N and three samples for total chromium and lead. The NH_3-N conentrations ranged from 1 to 148 mg/litre with a mean value of 60 mg/litre. Chromium concentrations ranged from 0.01 to 0.20 mg/litre and lead concentrations ranged from 0.20 to 0.24 mg/litre.

During the time of the Phase 3 study, seven measured NH_3-N concentrations ranged from 1 to 60 mg/litre with a mean value of 17 mg/litre. Chromium ranged from 0.01 to 0.06 mg/litre and lead ranged from 0.14 to 0.17 mg/litre. Shaul et al (7) reported on the concentrations of BOD, COD, TOC, and color made during the time of the present study.

DISCUSSION

Survival of the fathead minnows exposed to Phase 2 effluent was the most sensitive measure of effect in the ELS study. At the upper chronic limit of 10 percent, there was a significant (P=0.05) adverse effect on survival of the embryos to hatch. At effluent concentrations of 5 percent and less there were no effects on survival of embryos or larvae-juveniles, and no significant (P=0.05) effects on the growth of fish. Based on survival, the geometric mean of the chronic limits of 10 and 5 percent gives a chronic value (13) of 7.1 percent. Growth of the fathead minnows exposed to Phase 3 effluent was the most sensitive measure of effect in the chronic study. Survival of embryos and larvae-juveniles in all effluent concentrations was not significantly (P=0.05) less than that of control fish. However, the final mean weight of the juveniles exposed to 20 percent effluent was only 39 percent of that of control fish and this reduction in growth was significant (P=0.05). At effluent concentrations of 10 percent and less there were no effects on growth. Based on growth, the geometric mean of the chronic limits of 20 and 10 percent gives a chronic value of 14.1 percent.

An integrated use of chemical and toxicity measurements are often desirable to develop methods for a toxicity reduction evaluation on a wastewater treatment system. This study was designed to evaluate toxicity treatability of dyes and pigments wastewater treated in an AS treatment system dosed at two levels of PAC (1800 and 1000 mg/liter

effluent). The percentage of organics removed (7) was similar for these two dose levels. The acute and chronic toxicity of the effluent from the system dosed at the higher level of PAC was more toxic than the effluent from the system receiving the lower dosage of PAC. However, the dyes and pigments wastewater to the Phase 2 system was more toxic than that to the Phase 3 system with a 96-h LC_{50} value of 7 and 13 percent, respectively (7).

The 96-h LC_{50} value of the Phase 2 system dosed at 1800 mg/l averaged 31.2 percent and ranged from 12.9 to 80 percent. The effluent from the Phase 3 system was less toxic with 96-h LC_{50} values that averaged 100 percent and ranged from 28 to 100 percent. Using the average 96-h LC_{50} values of 31.2 percent for the Phase 2 effluent and 7 percent for the influent gives a toxicity reduction of 81 percent. Using the average 96-h LC_{50} values of 100 percent for the Phase 3 effluent and 13 percent for the influent gives a toxicity reduction of 87 percent. Thus the acute toxicity reduction was similar for both levels of PAC treatment. Also reduction of acute influent toxicity to chronic effluent toxicity was similar for both systems. For the Phase 2 system the influent acute value was 7 percent and the effluent chronic value was 7.1 percent. For the Phase 3 system the acute value of the influent was 13 percent and the chronic value of the effluent was 14.1 percent.

The primary function of PAC in a treatment system is the removal of dissolved organics. However, most inorganics, such as heavy metals and ammonia, are not removed by carbon. The removal and measurements of BOD, COD, TOC, and color (7) for the effluent for the Phase 2 and Phase 3 studies were similar, and these conventional organics are not correlated with the variation of acute toxicity. During the time of the present study, chromium and lead were the only heavy metals measured. These concentrations were not high enough to be toxic to the fathead minnow (13) and do not correlate with acute toxicity. During the Phase 2 acute tests, the chromium and lead concentrations for two of the samples were the same, but the acute values were different being 14.1 and 80 percent. During the Phase 2 exposure, ten analysis for NH_3-N were made of the effluent. The mean concentration was 60 mg/litre, and the values ranged from 1 to 148 mg/litre. During the Phase 3 exposure seven analysis for NH_3-N were made. The mean concentration was 17 mg/litre, and the values ranged from 1 to 64 mg/litre. Unfortunately, the time of sampling for the NH_3-N measurements and the initiation of the acute tests were different. However, the trend of the acute toxicity values and the measured NH_3-N values indicate that when NH_3-N values were high, toxicity was high (LC_{50} values low) and when NH_3-N values were low, toxicity was low. For example, the acute test initiated on December 21 gave an LC_{50} value of 14.1 percent, and the measured NH_3-N concentrations were 129.5 and 148 mg/litre on December 20 and 22, respectively. For the acute test initiated on December 14 the acute value was 80 percent and the measured NH_3-N concentrations were 0.8 and 3.46 mg/litre on December 13 and 16 respectively. The measured

concentrations of NH_3-N were higher for the Phase 2 study than for the Phase 3 study and the LC_{50} values and chronic value of the effluent from the Phase 2 study were lower (toxicity greater) than for the Phase 3 study.

Until recently effluent toxicity testing has been limited to acute tests to detect potential problems for the control of wastewater discharges. Acute toxicity tests do not indicate concentrations of an effluent that are "safe" under conditions of long-term exposure. Hart et al (14) discussed factors of safety for estimating permissible or safe concentrations of industrial wastes, chemicals, and other substances to freshwater fishes on the basis of acute toxicity tests. They suggested a tentative factor of 0.25 for estimating no-effect lethal concentrations from an acute test with a moderately sensitive species and when only the 96-h LC50 can be estimated. Henderson (15) discussed application factors to be applied to bioassay results for the safe disposal of toxic effluents and tentatively suggested a factor of 0.1. Mount and Stephan (16) carried out this concept further and proposed an application factor based on MATC of a chronic test divided by the 96-h LC50 value for the same species. This application factor was based on a single toxicant at constant concentration exposures. Water quality criteria (17) recommends that when an experimentally derived factor has not been determined concentrations of materials that are nonpersistent or have

nonaccumulative effects should not exceed 0.1 of the 96-h LC_{50} at any time or place after mixing with the receiving water. For toxicants that are presistent or cumulative the corresponding value is 0.05. The application factor (16) was modified and used in the water quality documents (13). In these documents the geometric mean of the MATC became the chronic value, and instead of an application factor (MATC/96-h LC_{50}) the acute/chronic ratio (96-h LC_{50}/chronic value) was used.

Many acute/chronic ratios have been developed for single toxicants using constant concentrations for both the acute and chronic exposure. However, there is very little published information on acute/chronic ratios for complex wastewaters with fluctuating outputs of various components of the waste. Thus it is possible that the components of the wastewater that causes acute toxicity may be different than the components that cause chronic toxicity. Woltering (18) lists chronic values for nine effluents, but in several of these studies it is not possible to calculate an acute/chronic ratio. Walden (19) reviewed the literature on the toxicity of pulp and paper mill effluents and evaluated various threshold sublethal effects in relation to 96-h LC_{50} values. He concluded that for kraft waste threshold concentrations for various sublethal parameters were between 0.05 and 0.1 of the 96-h LC_{50} values which corresponds to the modified acute/chronic ratio of from 20 to 10. DeGraeve et al (20) studied the toxicity of an underground coal gasification condenser water to the fathead minnow and found an acute value of 0.11 percent and a MATC value of .01 - 0.005 percent which gives

an acute/chronic ratio of 15.6. Iwan and Cella (21) used the fathead minnow to evaluate the toxicity of a treated municipal waste containing industrial effluents. They estimated an application factor of 0.2 and 0.4 which is equivalent to an acute/chronic ratio of 3.7. Rowe et al (22) used the flagfish to determine the life cycle chronic toxicity of treated liquid effluent from a petroleum refinery, and they found a threshold concentration of 9 percent. The Canadian guidelines recommends that the effluent is not acceptable if more than 50 percent of rainbow trout die, so they used this species in the acute tests. For the 30 acute tests mortality of trout in 100 percent effluent averaged five percent. If the flagfish is not more sensitive than the trout the acute value would be 100 percent giving an acute/chronic ratio of 11. Pickering (23) used ELS studies to determine the chronic toxicity to the fathead minnow of wastewater from a conventional treatment system receiving organic priority pollutants. The acute/chronic ratios were 6.6 for the unchlorinated effluent and 19 for the chlorinated-dechlorinated effluent.

The effluent from the Phase 2 system had a chronic value of 7.1 percent, and the acute value of the combined data is 31.2 percent. Thus the acute/chronic ratio is 4.4. Using the most toxic and least toxic LC_{50} values the acute/chronic ratios are 1.8 and 11.3 percent, respectively. The effluent from the Phase 3 system had a chronic value of 14.1 percent. The data from the combined results of the acute tests indicated an acute value of 80 percent and at this conentration, survival was 57 percent. Thus the acute value is not much greater than

80 percent. If the acute value is 100 percent, the acute/chronic ratio is 7.1 percent. Using the most toxic and least toxic LC_{50} values the acute/chronic ratios are 1.8 and 7.1 percent, respectively. These acute/chronic ratios for treated dyes and pigments processing wastewater are similar. The application factor of 0.1 (16) (acute/chronic ratio of 10) may be sufficient to predict "safe" long-term safe environmental concentrations from the acute toxicity data of this category of wastewater.

Most acute/chronic ratios have been developed on the basis of a constant acute value. In many complex wastewaters there is a fluctuating output of various components of the waste along with fluctuating acute values (1,24). In the present study the acute toxicity of the Phase 2 effluent ranged from 12.9 to 80 percent, and in the Phase 3 study ranged from 28.3 to 100 percent. Thus if an acute/chronic ratio is going to be used for predicting "safe" concentrations it is critical to know the variations in the acute toxicity of the wastewaters. For both of these studies the LC_{50} value of the most toxic sample was about two times the chronic value; maximum acute toxicity was similar to chronic toxcity.

The effectiveness of a wastewater treatment system may be evaluated by both chemical and toxicological parameters and, if so, should include the variability of these parameters. The acute toxicity test is an efficient means of quantifying the toxicity of a wastewater. The ultimate goal in regulating a wastewater is to prevent adverse impacts on the aquatic environment. A chronic toxicity test will integrate the

toxicity of a complex wastewater that varies in composition and concentration and is the most accurate test for estimating long-term "safe" concentrations for that species. However, chronic toxicity tests are longer and more expensive than acute tests. The short-term tests (4,5) for estimating chronic toxicity should add much information to the data base of chronic toxicity of wastewaters. However, until more chronic toxicity data of wastewaters is available an application factor of 0.1 (acute/chronic ratio of 10) applied to acute toxicity data of a dyes and pigments wastewater to estimate chronic "no-effect" concentrations appears to be a reasonable factor. With a few common and highly toxic chemicals, such as ammonia, it may be possible to evaluate and regulate wastewaters on a chemical basis to assess toxicity of the wastewater.

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TABLE 1 - SURVIVAL AND GROWTH OF EARLY LIFE-STAGES OF FATHEAD MINNOWS EXPOSED TO VARIOUS CONCENTRATIONS OF EFFLUENT FROM PHASE 2 TREATMENT (1800 MG PAC/LITRE).

ITEM	EFFLUENT CONCENTRATION (%) 10	5	2.5	1.3	0.7	CONTROL
Survival of Embryos at Test Day 2 (%)	94	94	93	95	95	90
Survival of Larvae From Test Day 2 to Hatch (%)	14[a]	100	100	100	100	100
Normal Larvae At Hatch (%)	0	100	100	100	100	100
Survival of 28 Day Old Fish From Test Day 2 (%)	0	93	93	97	93	95
Final Weight of 28 Day Old Fish Mean ± S.D. (mg)		102±24	100±25	100±29	116±20	111±20

a = Significantly different (P=0.05) from control.

TABLE 2 - SURVIVAL OF JUVENILE FATHEAD MINNOWS IN 96-HOUR, STATIC ACUTE EXPOSURES TO EFFLUENTS FROM PHASE 2 TREATMENT (1800 MG PAC/LITRE).

Effluent Concentration (%)	% SURVIVAL AND DATE OF TEST										TOTAL SURVIVAL (%)
	11/30	12/3	12/7	12/10	12/14	12/17	12/21	12/24	12/28	12/31	
80	0	0	0	0	100	100	0	0	0	0	20
40	0	0	0	0	100	100	0	0	100	60	36
20	0	0	100	100	100	100	0	0	100	100	60
10	100	90	100	100	100	100	100	100	100	100	99
5	100	100	100	100	100	100	100	100	100	100	100
Control	100	100	100	100	100	100	100	100	100	100	100
LC50(%)	14.1	12.9	28.3	28.3	>80	>80	14.1	14.1	47.6	42.3	31.2
95% Confidence Limits	10.6	9.5	21.2	21.2			10.6	10.6	41.0	41.0	28.0
	18.9	17.1	37.8	37.8			18.9	18.9	57.2	57.2	34.8

TABLE 3 - SURVIVAL AND GROWTH OF EARLY LIFE-STAGES OF FATHEAD MINNOWS EXPOSED TO VARIOUS CONCENTRATIONS OF EFFLUENT FROM PHASE 3 TREATMENT (1000 MG PAC/LITRE).

ITEM	EFFLUENT CONCENTRATION (%)					
	20	10	5	2.5	1.25	CONTROL
Hatchability of Embryos (%)	92	90	90	93	90	94
Normal Larvae At Hatch (%)	100	100	100	100	100	99
Survival of 21 Day Old Fish (%)	88	95	98	98	100	98
Final Weight (mg) Mean ± S.D. of 21 Day Old Fish	41±14	86±15	87±23	95±22	106±20	106±30

a = Significantly different (P=0.05) from control.

TABLE 4 - SURVIVAL OF JUVENILE FATHEAD MINNOWS IN 96-HOUR, STATIC-ACUTE EXPOSURES TO EFFLUENTS FROM PHASE 3 TREATMENT (1000 MG PAC/LITRE).

EFFLUENT CONCENTRATIONS (%)	% SURVIVAL AND DATE OF TEST							TOTAL SURVIVAL (%)
	2/4	2/8	2/11	2/15	2/18	2/22	2/25	
100	100	0	-	-	100	0	100	-
80	100	0	0	100	100	0	100	57
40	100	0	20	100	100	0	100	63
20	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100
CONTROL	100	100	100	100	100	100	100	100
LC50 (%)		>100	28.3	33.5	>80	>100	28.3	>100
95% Confidence Limits								
Lower			21.2	25.5			21.2	
Upper			37.8	46.2			37.8	